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Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat

C.-G. Chu · S. Chao · T. L. Friesen · J. D. Faris · S. Zhong · S. S. Xu

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Abstract Durum wheat (*Triticum turgidum* L. subsp. *durum*, 2n = *4x* = 28, AABB) is an important cereal used for making pasta products. Compared with bread wheat, durum wheat receives less attention in genetic and genomic studies. In this research, a tetraploid wheat doubled haploid (DH) population derived from the cross between the durum wheat cultivar 'Lebsock' and the *T. turgidum* subsp. *carthlicum* (2n = *4x* = 28, AABB) accession PI 94749 was developed. The population consisted of 146 lines and was used to construct linkage maps of all 14 chromosomes. The maps consisted of 280 SSR markers and spanned 2,034.1 cM with an average density of one marker per 7.2 cM. The DH population and the whole genome linkage maps were then used to identify QTLs associated with tan spot resistance. The DH population was inoculated separately with two Ptr ToxA-producing isolates (Pti2 and 86-124) representing races 1 and 2, respectively, of *Pyrenophora tritici-repentis*, and five resistance QTLs were detected on chromosome arms 3AS, 3BL, 5AL and 7BL. Together, the QTLs explained a total of 46 and 41% of the phenotypic variation for reaction to Pti2 and 86-124, respectively. The *Tsn1*-Ptr ToxA interaction was not a significant factor in tan spot development in this population, and none of the QTLs corresponded to previously identified loci known to confer insensitivity to host-selective toxins (HSTs) produced by *P. tritici-repentis*. This result, together with those of other similar studies, indicates that the wheat–*P. tritici-repentis* pathosystem involves more factors than currently published host-toxin interactions. The DH population and genetic maps reported here will be useful for genetic dissection of important agronomic traits as well as the identification and development of markers for marker-assisted selection (MAS).

Keywords *Triticum turgidum* · Durum wheat · Simple sequence repeat · *Pyrenophora tritici-repenti* · Tan spot

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C.-G. Chu · S. Zhong
Department of Plant Pathology, North Dakota State University, Fargo, ND 58105, USA

S. Chao · T. L. Friesen · J. D. Faris · S. S. Xu (✉)
Cereal Crops Research Unit, Northern Crop Science Laboratory, USDA-ARS, 1307 18th Street North, Fargo, ND 58105-5677, USA
e-mail: steven.xu@ars.usda.gov

Introduction

Genetic linkage maps are powerful tools for gene tagging, genome characterization, quantitative trait loci (QTL) analysis, evolutionary studies, and marker development for marker-assisted selection (MAS). Genetic maps are particularly useful for crops such as

hexaploid common wheat (*Triticum aestivum* L. 2n = *6x* = 42, AABBDD) and tetraploid durum wheat (*Triticum turgidum* L. subsp. *durum*, 2n = *4x* = 28, AABB) that have relatively large genomes.

To date, great efforts have been placed on the construction of whole genome linkage maps in hexaploid wheat (Chalmers et al. 2001; Chu et al. 2008c; Liu et al. 2005; Paillard et al. 2003; Quarrie et al. 2005; Somers et al. 2004; Song et al. 2005; Sourdille et al. 2003; Torada et al. 2006), but a relatively limited number of whole genome maps have been constructed in durum wheat (Blanco et al. 1998; Elouafi and Nachit 2004; Korzun et al. 1999; Maccaferri et al. 2008; Mantovani et al. 2008; Nachit et al. 2001; Peleg et al. 2008). Although both hexaploid and tetraploid wheat possess the A and B genomes, the two genomes vary somewhat between the two ploidy levels due to independent evolution over several thousand years (Chalupska et al. 2008). Furthermore, durum wheat is widely cultivated in the Mediterranean Basin, and also in Canada, the United States, Argentina and India, and it is an important cereal used for making pasta, couscous, burghul and other products (Nachit 1992). Therefore, the development of additional linkage maps in tetraploid wheat will provide essential resources and tools for genetic studies, breeding, and durum wheat improvement.

In the past decade, various types of molecular markers have been used to construct whole genome maps in wheat. Due to the ease of use, microsatellite (or simple sequence repeat; SSR) markers have been used extensively for the development of whole genome linkage maps in hexaploid wheat (Chalmers et al. 2001; Chu et al. 2008c; Liu et al. 2005; Paillard et al. 2003; Somers et al. 2004; Song et al. 2005; Sourdille et al. 2003; Torada et al. 2006). Korzun et al. (1999) demonstrated that SSRs from hexaploid wheat provide an excellent source of molecular markers for genetic studies and breeding of durum wheat.

Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. [anamorph *Drechslera tritici-repentis* (Died.) Shoem.], is a destructive foliar disease of both hexaploid and tetraploid wheat (Ali and Francl 2003). Most studies involving the genetic analysis of tan spot resistance has been conducted in hexaploid wheat, and in many cases, resistance corresponded to loci conferring insensitivity to host-selective toxins (HSTs) produced by *P. tritici-repentis*. For example, the *Tsn1* locus, which maps to the long arm of chromosome 5BL (Faris et al. 1996) confers sensitivity to Ptr ToxA, and the *Tsn1*-Ptr ToxA interaction has been shown to play a major role in conferring disease (Chu et al. 2008b; Friesen et al. 2003; Lamari and Bernier 1989b). Similarly, Friesen and Faris (2004) reported a significant association between tan spot resistance and the Ptr ToxB insensitivity gene *Tsc2* located on the short arm of chromosome 2B, and insensitivity to Ptr ToxC conferred by the *Tsc1* gene on chromosome arm 1AS was reported to be associated with resistance as well (Effertz et al. 2002). However, resistance loci beyond the known HST insensitivity genes have also been reported (Chu et al. 2008b; Faris and Friesen 2005).

Tan spot resistance in durum wheat and other tetraploid wheat subspecies has been investigated (Chu et al. 2008a, Singh et al. 2006b), but, except for one, the chromosome locations of the genes conferring resistance have not been identified (Singh et al. 2006a). In this study, we used two tetraploid wheat lines to develop a doubled haploid (DH) population and constructed linkage maps covering all 14 chromosomes. The DH population was then screened for reaction to two Ptr ToxA-producing isolates of *P. tritici-repentis* to determine if the *Tsn1*-Ptr ToxA interaction was an important factor in the development of tan spot, and to identify novel tan spot resistance QTLs.

Materials and methods

Plant materials

Accession PI 94749 of *T. turgidum* subsp. *carthlicum* and the durum wheat cultivar 'Lebsock' were used to develop a population consisting of 146 DH lines. PI 94749 was originally obtained from the USDA-ARS National Small Grain Research Facility, National Small Grain Collection, Aberdeen, ID. PI 94749 harbors resistance to Fusarium head blight (FHB) (Oliver et al. 2008) and Stagonospora nodorum blotch (SNB), but was moderately susceptible to tan spot (Chu et al. 2008a). Lebsock was developed by the North Dakota Agricultural Experiment Station in cooperation with USDA-ARS and was officially released in 1999. Lebsock harbors resistance to stem rust and leaf rust, is moderately resistant to tan spot, and is moderately susceptible to FHB (Elias et al. 2001).

The DH population was developed using the wheat × maize method described in Chu et al. (2008c). All 146 DH lines were used to construct linkage maps and conduct QTL analysis for tan spot resistance.

SSR marker generation

Genomic DNA was isolated using the method described by Dvorak et al. (1988). The DNA concentration was adjusted to 30–50 ng/μl for PCR reactions. Based on previous surveys for polymorphism between PI 94749 and Lebsock, a total of 281 SSR primer sets including 87 BARC (Song et al. 2005), 64 GWM (Röder et al. 1998a, b), 3 GDM (Pestsova et al. 2000), 17 CFA (Sourdille et al. 2003), 9 CFD (Guyomarc'h et al. 2002), 90 WMC (Somers et al. 2004) and 11 DuPw (Eujayl et al. 2002) were used to generate markers for developing linkage maps in the DH population. SSR fragments were amplified by PCR following the conditions described in Somers et al. (2004). SSRs were separated by capillary electrophoresis using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA) using the procedures described in Chao et al. (2007).

Linkage map construction

The computer program QGENE (Nelson 1997) was used to test the segregation ratios of all markers for fit to the expected 1:1 ratio by Chi-squared analysis. The computer program MAPMAKER (V2.0) for Macintosh (Lander et al. 1987) was then used to perform linkage analysis with a minimum LOD threshold of 3.0 and the Kosambi mapping function (Kosambi 1944). Several large linkage groups were first obtained by using the ''two-point/group'' command with a minimum LOD = 3.0 and a maximum $\theta = 0.40$, and then each large group was regrouped using a minimum of LOD = 10.0. The FIRST ORDER and RIPPLE (LOD > 3.0) commands were used to determine the most plausible order of markers within linkage groups. The TRY command was used to add markers that did not RIPPLE at an LOD > 3.0 to the established framework maps. Positions of centromeres on the maps were estimated according to the published physical map (Sourdille et al. 2004).

Tan spot resistance evaluation

Pyrenophora tritici-repentis isolates Pti2 (race 1) (Friesen et al. 2002) and 86-124 (race 2) (Friesen et al. 2003) were selected for this study because they are the two most common races in North America and both produce Ptr ToxA (Tomás and Bockus 1987; Lamari and Bernier 1989b; Tuori et al. 1995). Disease inoculations were conducted in three replicated experiments under controlled conditions using procedures described in Friesen et al. (2003), and all plants were inoculated with conidia of each isolate separately. Totally, nine plants were analyzed collectively for each replicate and a total of three replicates were used for the entire population with each isolate.

Fungi were grown and conidia were harvested as described by Lamari and Bernier (1989a). Spore inoculum was adjusted to 3,000 spores/ml, and two drops of Tween-20 (Sigma, Saint Louis, MI) were added per 100 ml of inoculum. Plants were inoculated until runoff and placed in 100% relative humidity in the light at 21°C for 24 h, and then placed in a growth chamber under a 12-h photoperiod at 21°C. Reaction types were scored 7 days post inoculation using the 1–5 reaction type rating scale developed by Lamari and Bernier (1989a), where 1 = resistant, 2 = moderately resistant, 3 = moderately resistant to moderately susceptible, 4 = moderately susceptible, and 5 = susceptible. Lines showing equal numbers of two reaction types were given an intermediate score (e.g. lines showing equal numbers of reaction type 1 and 2 were scored as 1.5).

PI 94749 is insensitive to Ptr ToxA (Chu et al. 2008a), and Lebsock was sensitive in preliminary experiments. To investigate Ptr ToxA reaction in the DH population, leaves of each line were infiltrated with purified Ptr ToxA at the two-leaf stage as described in Chu et al. (2008c). Purified Ptr ToxA was provided by S.W. Meinhardt, Department of Plant Pathology, North Dakota State University, Fargo, ND. Infiltrated plants were scored 4 days after infiltration as sensitive or insensitive based on the presence or absence of necrosis.

Statistical and QTL analysis for tan spot resistance

Bartlett's χ^2 was calculated to test for homogeneity of variances among different experiments (SAS Institute

1999). The mean scores of all lines were obtained by combining data from homogeneous experiments and used for statistical and QTL analysis. Mean scores based on Ptr ToxA sensitivity were compared using Fisher's protected least significant difference (LSD) at $P < 0.05$ (SAS Institute 1999) to test the significance of the *Tsn1*-Ptr ToxA interaction in tan spot development. For QTL detection, single-factor regression analysis was performed using the entire marker data set, but for simple interval mapping (SIM) and composite interval-regression mapping (CIM), a subset of 188 markers spaced >2 cM apart and giving the most complete genome coverage was used. QTL analysis was performed using the program Windows QTL Cartographer (v2.5) (Wang et al. 2007). A permutation test with 5,000 permutations indicated that a LOD threshold of 3.10 in this population yields an experiment-wise significance level of 0.05. Markers with the most significant effect for each QTL for a given trait were assembled into multiple regression models (Nelson 1997), and the coefficient of determination (R^2) from the multiple regression model is the total amount of phenotypic variation explained by the markers. Markers with significant ($P < 0.001$) main effects were tested against all other markers (Manly et al. 2001) in the dataset to identify significant ($P < 10^{-6}$) interactions among QTLs.

Results

Genetic linkage mapping

A total of 290 loci were generated by 281 SSR primer pairs, and ten of them were either unlinked or formed small linkage groups that could not be assigned to chromosomes and thus were eliminated from the data set. Therefore, 280 SSR marker loci were used to assemble the 14 linkage groups (Fig. 1, Table 1). Linkage groups were anchored to specific chromosomes according to published genetic and physical maps (Somers et al. 2004; Sourdille et al. 2003). The total genetic distance of the linkage maps was 2,034.1 cM, with an average density of one marker per 7.2 cM.

The total map lengths were 1,153.4 and 880.7 cM for the A and B genomes, respectively, (Table 1). The B genome contained 148 marker loci with an average of 6.0 cM per locus and was more dense than the A genome, which had 133 marker loci with a density of 8.7 cM per marker. Of the 280 marker loci, 35 (12.5%) had segregation ratios that deviated significantly from the expected 1:1 ratio and were located on eight chromosomes (1A, 1B, 2B, 3B, 4A, 5A, 6A, and 7A; Table 1; Fig. 1). For the most part, loci with distorted segregation ratios mapped near chromosome ends or in centromere regions (Fig. 1). Chromosomes 2B and 3B contained the most marker loci with skewed segregation ratios (9 and 10, respectively), and those on chromosome 3B were clustered in the long arm (Fig. 1).

Map lengths for individual chromosomes ranged from 85 (chromosome 1B) to 191.6 cM (chromosome 3A) (Table 1). Six chromosomes (3A, 4A, 5A, 6A, 7A, and 7B) contained gaps greater than 30 cM (Fig. 1). Clustering of markers near centromeric regions was observed for all chromosomes, and clustering in other non-centromeric regions was also observed in most chromosomes (Fig. 1).

The orders and distances of most of the SSR markers on our maps are consistent with those in genetic maps developed by Somers et al. (2004), but we observed a major difference between the two maps on chromosome 1A, where the marker order in the segment delineated by *Xwmc24* and *Xbarc83* was reversed. However, our marker order in this segment was consistent with that of the physical map published by Sourdille et al. (2004).

Tan spot evaluation in the DH population

Tan spot reaction types were recorded 7 day post-inoculation for each DH line. The data from the three replicated experiments for each isolate were homogeneous (Bartlett's $\chi^2_{df=2} = 1.96$ and 0.69, and $P = 0.38$ and 0.71 for reaction to isolates Pti2 and 86-124, respectively). Thus, reaction type data from three experiments for each isolate were combined separately and the means were used for statistical analysis and QTL identification. PI 94749 had an average reaction type of 3.5 and was moderately susceptible to both isolates, whereas Lebsock showed a mean reaction type of 2.5 and was moderately resistant to the two isolates.

In the DH population, reaction types for Pti2 ranged from 1.3 to 4.2 with an average of 3.0, and for reaction to 86-124, reaction types ranged from 1.0 to

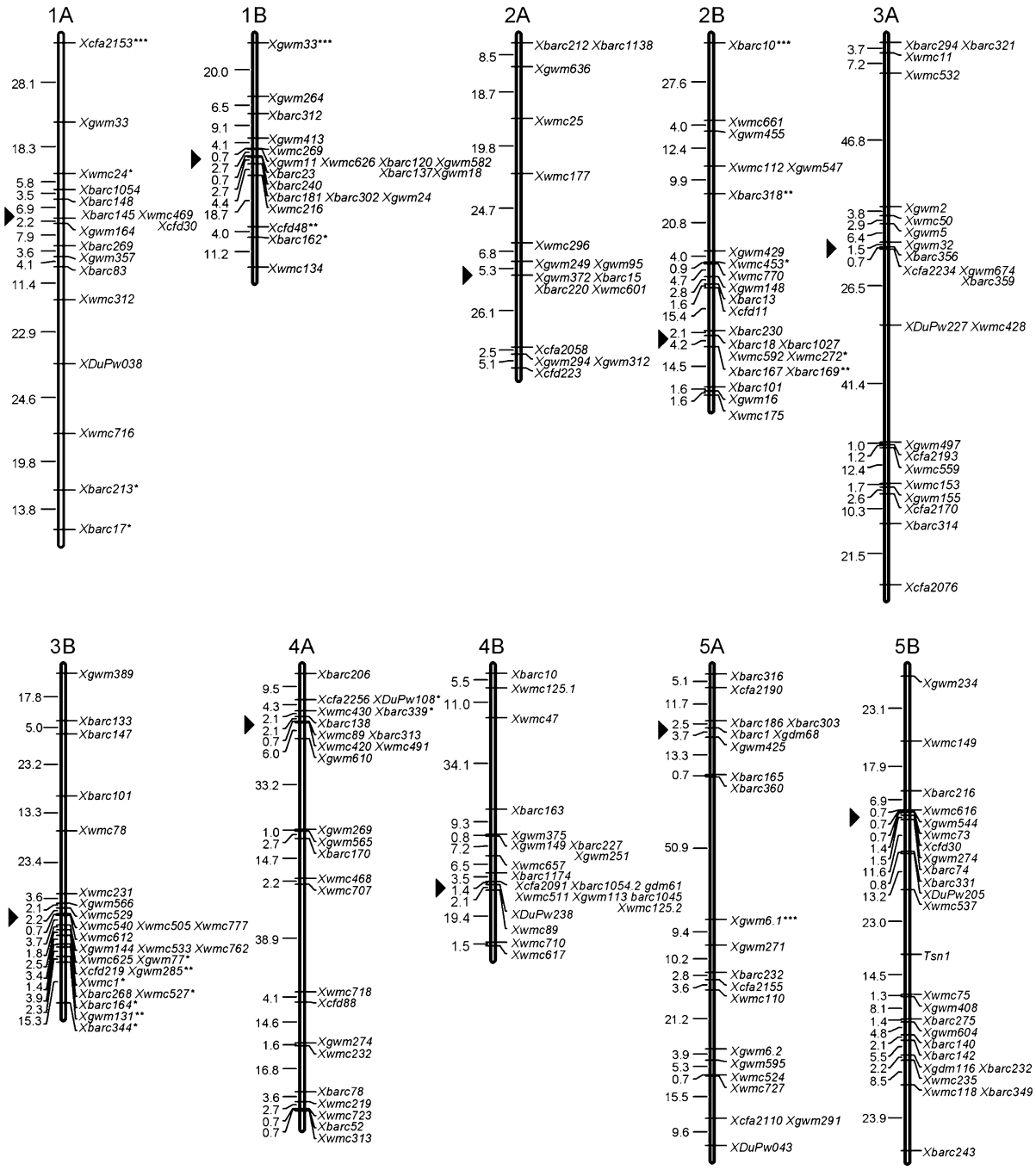

Fig. 1 Genetic linkage maps constructed in the Lebsock × PI 94749 derived DH population. The positions of marker loci are shown to the *right* of the linkage groups and centiMorgan (cM) distances between loci are shown along the *left*. *Black triangles* indicate the positions of centromeres estimated according to the physical map of Sourdille et al. (2004). Markers with significantly skewed segregation ratios are indicated with asterisks where *, **, and *** indicates the marker segregation distortion was significant at $P < 0.05$, 0.01 and 0.001, respectively

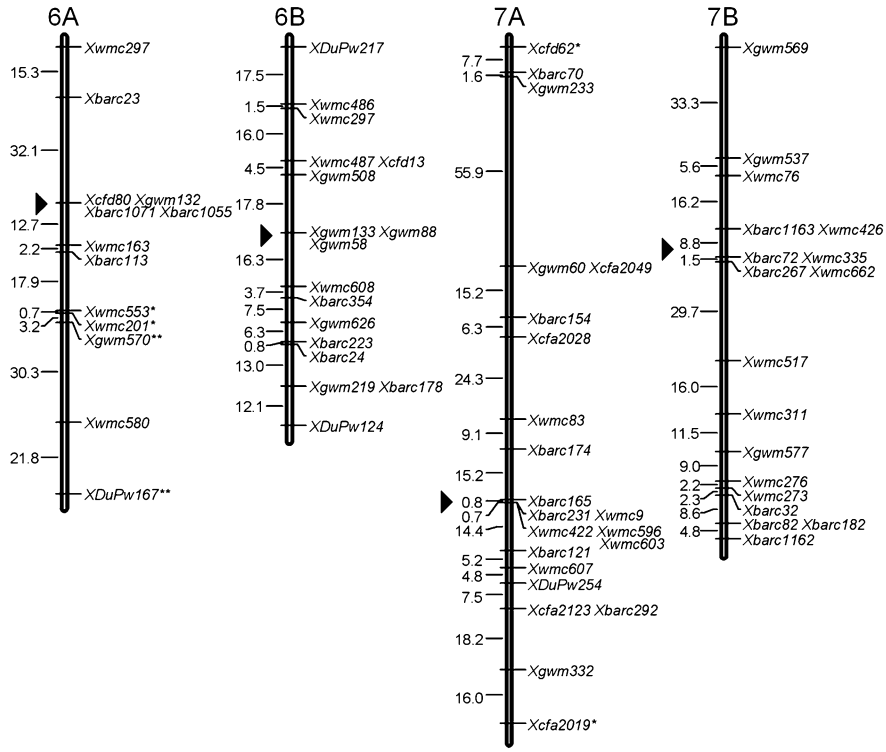

Fig. 1 continued

Table 1 Chromosome assignment and marker distribution, genetic length, and marker density of the linkage maps constructed in the Lebsock × PI 94749 derived DH population

Chromosome	Marker number	Genetic length (cM)	Marker density (cM/marker)	No. markers with distorted ratios
1A	17	173.1	10.2	4
1B	20	85	4.3	3
2A	16	117.5	7.3	0
2B	22	127.9	5.8	9
3A	22	191.6	8.7	0
3B	25	125.5	5.0	10
4A	22	162.1	7.4	2
4B	21	102.3	4.9	0
5A	21	170.3	8.1	1
5B	25	173.6	6.9	0
6A	13	136.1	10.5	4
6B	17	116.9	6.9	0
7A	22	202.7	9.2	2
7B	18	149.5	8.3	0
A genome	133	1153.4	8.7	13
B genome	148	880.7	6.0	22
Total	281	2034.1	7.2	35

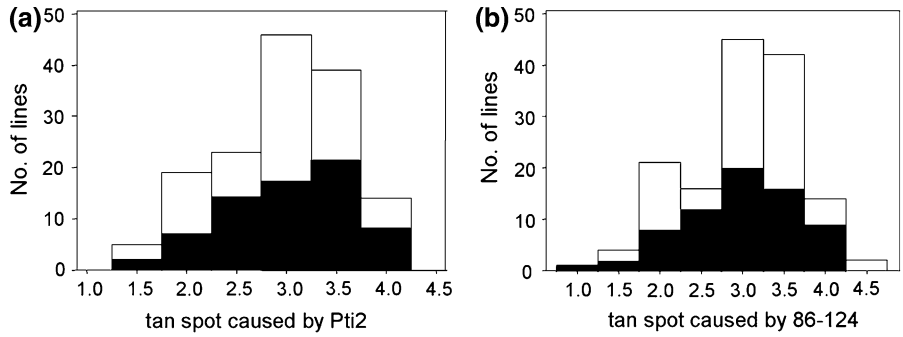

Fig. 2 Histograms of the Lebsock × PI 94749 derived DH population distribution for reaction to spore inoculation with isolates Pti2 (race 1) (a) and 86-124 (race 2) (**b**). Ptr ToxA insensitive lines are indicated in *black*

4.5 with an average of 3.0. Overall, the distribution of tan spot caused by Pti2 and 86-124 indicated that resistance in the population was quantitatively controlled, and transgressive segregation was observed (Fig. 2). The population segregated 69 insensitive: 77 sensitive for reaction to Ptr ToxA, which fit the expected 1:1 segregation ratio ($\chi^2_{df=1} = 0.44$, $P = 0.51$), and the *Tsn1* locus that confers Ptr ToxA sensitivity was mapped on chromosome arm 5BL (Fig. 1), the same position as seen previously (Faris et al. 1996). The distribution of reaction types for isolates Pti2 and 86-124 in insensitive DH lines is shown in Fig. 2. The mean reaction types of Ptr ToxA insensitive and sensitive DH lines were 3.0 and 2.9 for reaction to Pti2, and 3.0 and 3.0 for reaction to 86-124, respectively. The two reaction type means were not significantly different ($LSD_{0.05} = 0.21$ and 0.22 for reaction to isolates Pti2 and 86-124, respectively). This result demonstrates that a compatible *Tsn1*-Ptr ToxA interaction does not play a significant role in tan spot development in this population.

QTL detection for tan spot resistance

The single-factor regression analysis indicated that none of the ten unlinked markers were associated with tan spot resistance. However, five QTLs identified by the mapped markers were significantly associated with tan spot resistance in the DH population (Table 3, Fig. 3). Two of the five QTLs were located on chromosome arm 5AL (designated as *QTs.fcu-5A.1* and *QTs.fcu-5A.2*), and the remaining three were on chromosome arms 3AS (*QTs.fcu-3A*), 3BL (*QTs.fcu-3B*) and 7BL (*QTs.fcu-7B*). PI 94749 contributed the resistance effects of QTLs *QTs.fcu-5A.2* and *QTs.fcu-7B*, and Lebsock contributed the resistance alleles for the other three QTLs. The total phenotypic variation explained by the five QTLs in the multiple regression model was 46 and 41% for reaction to isolates Pti2 and 86-124, respectively (Table 2). No significant interactions among the QTLs were detected.

Although the five QTLs showed the same peak position for reaction to isolates Pti2 and 86-124, the significance of each QTL varied when inoculated with different isolates (Table 3; Fig. 2). For reaction to isolate Pti2, *QTs.fcu-5A.1* and *QTs.fcu-3A* were the two most significant QTLs and explained 22 and 11% of the phenotypic variation, respectively. *QTs.fcu-5A.1* was also the most significant QTL for reaction to isolate 86-124 explaining 15% of the trait variation, but *QTs.fcu-3A* had relatively minor effects and explained only 8% of the phenotypic variation. The effects of *QTs.fcu-5A.2* were increased for reaction to isolate 86-124 and it explained 13% of the phenotypic variation. Compared to the reaction to Pti2, the significance of *QTs.fcu-3B* and *QTs.fcu-7B* were reduced greatly for reaction to 86-124. The LOD values for these two QTLs were 2.36 and 3.01, respectively, which indicates they were both below the critical LOD threshold of 3.10 (Fig. 3).

Discussion

The durum wheat cultivar Lebsock and the *T. turgidum* subsp. *cartlicum* accession PI 94749 differ widely in that Lebsock has superior agronomic and quality traits and has been the leading durum cultivar grown in North Dakota since 2004 (North Dakota Wheat Commission 2008), whereas PI 94749 is considered a wild relative with poor agronomic performance. The two genotypes are also known to have different reactions to several important diseases such as FHB

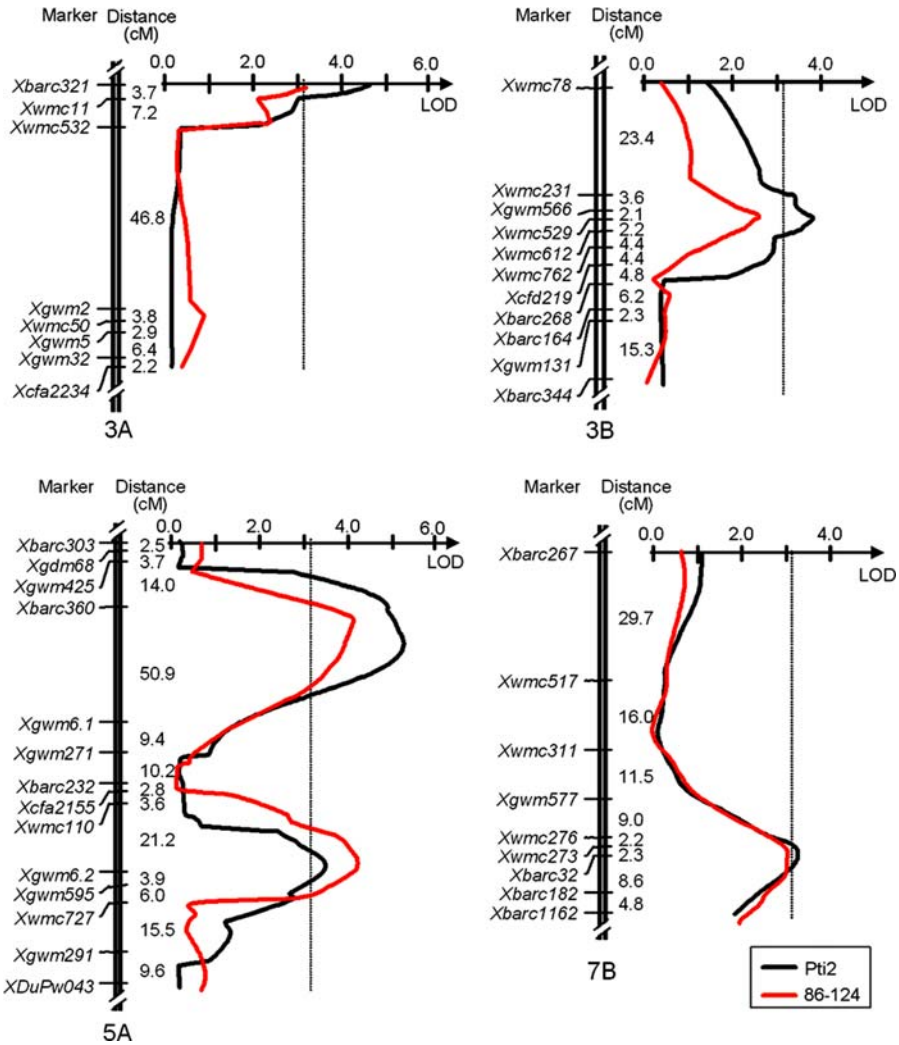

Fig. 3 Partial maps of chromosomes that carried QTL associated with tan spot resistance in composite interval mapping (CIM) in the DH population derived from Lebsock × PI 94749. The positions of marker loci are shown to the left of each linkage group and centiMorgan (cM) distances between loci are shown along the right. The *black* and *grey* lines indicate QTL associated with resistance to isolates Pti2 (race 1) and 86-124 (race 2), respectively. The vertical dotted line indicates the logarithm of the odds (LOD) significance threshold of 3.10. The LOD and R^2 values for each QTL are listed in Table 3

Table 2 Single-factor and multiple regression analysis of the markers significantly associated with tan spot resistance caused by isolates Pti2 (race 1) and 86-124 (race 2) of *P tritici-repentis* in the DH population derived from Lebsock × PI 94749

Significant marker (chromosome arm)	R^2 value	
	Pti2	86-124
Single-factor regression		
Xbarc321 (3AS)	0.12***	0.08**
Xwmc529 (3BL)	0.08**	0.05*
Xbarc360 (5AL)	0.24***	0.16***
Xgwm6.2 (5AL)	0.08**	0.14***
Xwmc273 (7BL)	0.07**	0.06*
Multiple regression	0.46***	0.41***

Significance levels: *** $P < 0.0001$, ** $P < 0.001$, * $P < 0.01$

(Oliver et al. 2008), SNB (Chu et al. 2008a), stem rust, and leaf rust (Elias et al. 2001). Therefore, the DH population derived from the cross of Lebsock × PI 94749 and the genetic linkage maps developed in this work will be useful tools and resources for targeting genomic regions associated with disease resistance as well as important agronomic and quality traits, and providing useful information and materials for genetic studies and breeding.

The total genetic distance of the whole genome linkage maps was 2,034.1 cM. Comparisons of our maps with previously published genetic maps in hexaploid wheat (Somers et al. 2004; Chu et al. 2008c) indicate that our maps have good coverage of most chromosomes. However, several chromosomes were only partially covered based on the estimated

Table 3 Composite interval mapping (CIM) analysis of QTLs associated with resistance to isolates Pti2 (race 1) and 86-124 (race 2) in the doubled haploid population derived from Lebsock × PI 94749

QTL	Marker interval[a]	Peak marker[b]	R^2 value		LOD		Additive effect[c]	
			Pti2	86-124	Pti2	86-124	Pti2	86-124
QTs.fcu-3A	*Xbarc321–Xwmc11*	*Xbarc321*	0.11	0.08	4.62	3.10	−0.44 L	−0.37 L
QTs.fcu-3B	*Xwmc231–Xwmc762*	*Xwmc529*	0.08	0.05	3.88	2.36	−0.36 L	−0.30 L
QTs.fcu-5A.1	*Xgwm425–Xgwm6.1*	*Xbarc360*	0.22	0.15	5.14	4.05	−0.52 L	−0.42 L
QTs.fcu-5A.2	*Xwmc110–Xgwm595*	*Xgwm6.2*	0.08	0.13	3.32	4.11	−0.31 P	−0.40 P
QTs.fcu-7B	*Xwmc276–Xbarc182*	*Xwmc273*	0.08	0.06	3.13	3.01	−0.36 P	−0.30 P

LOD logarithm of the odds

[a] Markers within the interval were significantly (LOD > 3.1) associated with resistance in CIM

[b] The most significant marker in the marker interval

[c] Additive main effect contributed by allele from corresponding parent, where L = Lebsock and P = PI 94749. The negative sign indicates the QTL allele showed a reduced effect on tan spot rate

centromere position from physical maps in Sourdille et al. (2004), particularly for the long arms of chromosomes 2A, 2B, 3B, and 4B. We estimate that our maps have about 75% coverage of the whole genome. Therefore, to increase the potential of identifying genes of interested, additional work is needed to develop more markers to increase the map coverage and to fill several large gaps.

Segregation distortion of markers is a common phenomenon that has been observed on previously published maps. In hexaploid wheat, the percentage of distorted markers ranged from 3 (Quarrie et al. 2005) to 27% (Cadalen et al. 1998). We observed that 12.5% of the markers showed skewed segregation ratios and chromosome 3B harbored the most markers with distorted segregation ratios, which were clustered near the centromeric region. The centromeric clustering of markers with distorted ratios was also observed by Chu et al. (2008c) on chromosome 4B in a map developed based on a hexaploid DH population. In the current work, chromosome arm 3BL marker segregation ratios were skewed in favor of PI 94749 alleles. It is possible that chromosome arm 3BL of PI 94749 harbors gene(s) that affect fertilization by maize pollen or increase the viability of haploid embryos during the embryo-rescuing process. It is also possible that PI 94749 harbors factors involved in competition among gametes for preferential fertilization as was shown to occur in tetraploid wheat by Kumar et al. (2007).

Resistance to tan spot caused by isolates of *P. tritici-repentis* races 1 and 2 in this population was governed by five genomic regions. Lebsock carried resistance alleles at the most QTLs with one major QTL on chromosome arm 5AL (*QTs.fcu-5A.1*) that was the most significant for reaction to both isolates. This is consistent with the observation that Lebsock was more resistant than PI 94749. The transgressive segregation of DH lines with better resistance than either parent indicated that these QTLs had some additive characteristics and could be combined to develop highly resistant germplasm. In addition, our results also indicated that the sources with a relatively lower level of resistance could still be used in breeding to greatly improve tan spot resistance.

Of the five QTLs identified in this population, *QTs.fcu-3B* may be the same as the one detected in hexaploid wheat by Faris and Friesen (2005) and in tetraploid wheat by Singh et al. (2006a) based on the chromosome positions of common markers. Also, *QTs.fcu-5A.1* and *QTs.fcu-3A* may be the same as those identified by Chu et al. (2008b) and Singh et al. (2008) in hexaploid wheat, respectively. The remaining two, *QTs.fcu-5A.2* and *QTs.fcu-7B*, were novel QTLs identified in this research, since the resistance conferred by these genomic regions has not been reported.

Insensitivity to Ptr ToxA has previously been shown to be associated with tan spot resistance in hexaploid wheat (Chu et al. 2008b; Friesen et al. 2003; Lamari and Bernier 1989b), but no significant association between disease resistance and Ptr ToxA insensitivity was found in our DH population. This

result is not unexpected because PI 94749 was insensitive to Ptr ToxA and yet was moderately susceptible to both Ptr ToxA-producing isolates. This is consistent with the results of Chu et al. (2008a) where the *Tsn1*-Ptr ToxA interaction was not significantly associated with tan spot development in a collection of 206 tetraploid wheat accessions. Also, Faris and Friesen (2005) demonstrated that the *Tsn1* locus was not significantly associated with tan spot resistance in a hexaploid wheat population. The non-association between Ptr ToxA insensitivity and disease resistance in this population might be due to the possibility that Ptr ToxA is partially contained and/or the *Tsn1*-Ptr ToxA interaction may be superseded by resistance mechanisms that limit the effects of the toxin, which would lead to host resistance regardless of toxin sensitivity as suggested by Faris and Friesen (2005). Further investigations are needed to evaluate the expression and government of the *Tsn1*-Ptr ToxA interaction in this population.

Pyrenophora tritici-repentis isolate 86-124 is known to produce Ptr ToxA, and isolate Pti2 is known to produce both Ptr ToxA and Ptr ToxC (Effertz et al. 2002). We did not evaluate reaction to the *P. tritici-repentis* toxin Ptr ToxC. However, evaluation of the population for reaction to Pti2 revealed no significant effects from the chromosome arm 1AS region, which is known to harbor the *Tsc1* gene that governs sensitivity to Ptr ToxC (Effertz et al. 2002). This result together with the fact that the *Tsn1*-Ptr ToxA interaction was not a significant factor in disease for either isolate makes it likely that 86-124 and Pti2 produce other virulence factors in addition to Ptr ToxA and/or Ptr ToxC which are important in disease induction. The DH population derived from Lebsock × PI 94749 may be useful for conducting further studies to characterize additional pathogen-related factors involved in causing tan spot.

In summary, we developed a tetraploid wheat DH population and constructed linkage maps spanning most of the genome. Five QTLs were identified for conferring tan spot resistance in this population, and two of them were novel. None of the QTLs corresponded to known HST insensitivity loci, and the *Tsn1*-Ptr ToxA interaction was not a significant factor for tan spot development in this population. The DH population and genetic maps developed in this study provided useful tools for genetic research as well as the identification and development of markers for marker-assisted selection (MAS) in durum wheat.

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References

Ali S, Francl LJ (2003) Population structure of *Pyrenophora tritici-repentis* prevalent on wheat and noncereal grasses in the Great Plains. Plant Dis 87:418–422

Blanco A, Bellomo MP, Cenci A, De Giovanni C, D'Ovidio R, Iacono E, Laddomada B, Pagnotta MA, Porceddu E, Sciancalepore A, Simeone R, Tanzarella OA (1998) A genetic linkage map of durum wheat. Theor Appl Genet 97:721–728

Cadalen T, Sourdille P, Charmet G, Tixier MH, Gay G, Boeuf C, Bernard S, Leroy P, Bernard M (1998) Molecular markers linked to genes affecting plant height in wheat using a doubled-haploid population. Theor Appl Genet 96:933–940

Chalmers KJ, Cambell AW, Kretschmer J, Karakousis A, Henschke PH, Pierens S, Harker N, Pallotta M, Cornish GB, Shariflou MR, Rampling LR, McLanchlan A, Daggard G, Sharp PJ, Holton TA, Sutherland MW, Appels R, Langridge P (2001) Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust J Agric Res 52:1089–1119

Chalupska D, Lee HY, Faris JD, Evrard A, Chalhoub B, Haselkorn R, Gornicki P (2008) Acc homoeoloci and the evolution of wheat genomes. Proc Natl Acad Sci USA 105:9691–9696

Chao S, Zhang W, Dubcovsky J, Sorrells M (2007) Evaluation of genetic diversity and genome-wide linkage disequilibrium among U.S. wheat (*Triticum aestivum* L.) germplasm representing different market classes. Crop Sci 47:1018–1030

Chu CG, Friesen TL, Faris JD, Xu SS (2008a) Evaluation of seedling resistance to tan spot and Stagonospora nodorum blotch in tetraploid wheat. Crop Sci 48:1107–1116

Chu CG, Friesen TL, Xu SS, Faris JD (2008b) Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theor Appl Genet 117:873–881

Chu CG, Xu SS, Friesen TL, Faris JD (2008c) Whole genome mapping in a wheat doubled haploid population using SSRs and TRAPs and the identification of QTL for agronomic traits. Mol Breed 22:251–266

Dvorak J, McGuire PE, Cassidy B (1988) Apparent sources of the A genomes of wheats inferred from the polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30:680–689

Effertz RJ, Meinhardt SW, Anderson JA, Jordahl JG, Francl LJ (2002) Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92:527–533

Elias EM, Miller JD, Manthey FA (2001) Registration of 'Lebsock' durum wheat. Crop Sci 41:2007–2008

Elouafi I, Nachit MM (2004) A genetic linkage map of the *durum* × *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. Theor Appl Genet 108:401–413

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor Appl Genet 104:399–407

Faris JD, Friesen TL (2005) Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor Appl Genet 111:386–392

Faris JD, Anderson JA, Francl LJ, Jordahl JG (1996) Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86:459–463

Friesen TL, Faris JD (2004) Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor Appl Genet 109:464–471

Friesen TL, Rasmussen JB, Ali S, Kwon CY, Francl LJ, Meinhardt SW (2002) Reaction to *Pyrenophora tritici-repentis* race 1 by wheat mutants insensitive to Ptr ToxA. Phytopathology 92:38–42

Friesen TL, Ali S, Kianian S, Francl LJ, Rasmussen JB (2003) Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. Phytopathology 93:397–401

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterization of polymorphic microsatellite markers from *Aegilops taushii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

Institute SAS (1999) SAS/STAT User's Guide, Releases: 8.2, 8.1, 8.0. SAS Institute, Inc., Cary

Korzun V, Röder MS, Wendekake K, Pasqualone A, Lotti C, Ganal MW, Blanco A (1999) Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. Theor Appl Genet 98:1202–1207

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kumar S, Gill BS, Faris JD (2007) Identification and characterization of segregation distortion loci along chromosome 5B in tetraploid wheat. Mol Genet Genomics 278:187–196

Lamari L, Bernier CC (1989a) Evaluation of wheat lines and cultivars for reaction to tan spot *Pyrenophora tritici-repentis* based on lesion size. Can J Plant Pathol 11:49–56

Lamari L, Bernier CC (1989b) Toxin of *Pyrenophora tritici-repentis*: host-specificity, significance in disease, and inheritance of host reaction. Phytopathology 79:740–744

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD (2005) A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor Appl Genet 111:782–794

Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Salem MB, Bort J, DeAmbrogio E, Garcia del Moral LF, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. Genetics 178:489–511

Manly KK, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross platform software for genetic mapping. Mamm Genome 12:930–932

Mantovani P, Maccaferri M, Sanguineti MC, Tuberosa R, Catizone I, Wenzl P, Thomson B, Carling J, Huttner E, DeAmbrogio E, Kilian A (2008) An integrated DArT-SSR linkage map of durum wheat. Mol Breed 22:629–648

Nachit MM (1992) Durum wheat breeding for Mediterranean dryland of North Africa and West Asia. In: Rajram S, Saari EE, Hetel GP (eds) Durum wheats: "challenges and opportunities". CIMMYT, Ciudad Obregon, Mexico, pp 14–27

Nachit MM, Elouafi I, Pagnotta MA, El Saleh A, Iacono E, Labhilili M, Asbati A, Azrak M, Hazzam H, Benscher D, Khairallah M, Ribaut JM, Tanzarella OA, Porceddu E, Sorrels ME (2001) Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. *durum*). Theor Appl Genet 102:177–186

Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

North Dakota Wheat Commission (2008) NDWC Releases Top Wheat Varities Grown in 2008. Available at http://www.ndwheat.com/growers/detail.asp?newsID=1218, posted on July 21, 2008, verified on March 25, 2009

Oliver RE, Cai X, Friesen TL, Halley S, Stack RW, Xu SS (2008) Evaluation of Fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). Crop Sci 48:213–222

Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An intergrative genetic linkage map of winter wheat (*Triticum aestivum* L.). Theor Appl Genet 107:1235–1242

Peleg Z, Saranga Y, Suprunova T, Ronin Y, Röder MS, Kilian A, Korol AB, Fahima T (2008) High-density genetic map of durum wheat × wild emmer wheat based on SSR and DArT markers. Theor Appl Genet 117:103–115

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti M-C, Hollington PA, Aragués R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880

Röder MS, Korzun V, Gill BS, Ganal MW (1998a) The physical mapping of microsatellite markers in wheat. Genome 41:278–283

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998b) A microsatellite map of wheat. Genetics 149:2007–2023

Singh PK, Gonzalez-Hernandez JL, Mergoum M, Ali S, Adhikari TB, Kianian SF, Elias EM, Hughes GR (2006a) Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. Phytopathology 96:885–889

Singh PK, Mergoum M, Ali S, Adhikari TB, Elias EM, Anderson JA, Glover KD, Berzonsky WA (2006b) Evaluation of elite wheat germplasm for resistance to tan spot. Plant Dis 90:1320–1325

Singh S, Bockus WW, Sharma I, Bowden RL (2008) A novel source of resistance in wheat to *Pyrenophora tritici-repentis* Race 1. Plant Dis 92:91–95

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Cadalen T, Guyomarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot-Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet 106:530–538

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Tomás A, Bockus WW (1987) Cultivar specific toxicity of culture filtrate of *Pyrenophora tritici-repentis*. Phytopathology 77:1337–1366

Torada A, Koike M, Mochida K, Ogihara Y (2006) SSR-based linkage map with new markers using an intraspecific population of common wheat. Theor Appl Genet 112: 1042–1051

Tuori RP, Wolpert TJ, Ciuffetti LM (1995) Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis*. Mol Plant Microbe Interact 8:41–48

Wang S, Basten CJ, Zeng ZB (2007) Windows QTL Cartographer 2.5. Department of Statistics. North Carolina State University, Raleigh